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Plant growth drives soil nitrogen cycling and N-related microbial activity through changing root traits

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Abstract :

Relationships between plants and nitrogen-related microbes may vary with plant growth. We investigated these dynamic relationships over three months by analyzing plant functional traits (PFT), arbuscular mycorrhizal fungal (AMF) colonization, potential N mineralization (PNM), potential nitrification (PNA) and denitrification activities (PDA) in *Dactylis glomerata* cultures. *D. glomerata* recruited AMF during early growth, and thereafter maintained a constant root colonization intensity. This may have permitted high enough plant nutrient acquisition over the three months as to offset reduced soil inorganic N. PFT changed with plant age and declining soil fertility, resulting in higher allocation to root biomass and higher root C:N ratio. Additional to root AMF presence, PFT changes may have favored denitrification over mineralization through changes in soil properties, particularly increasing the quality of the labile carbon soil fraction. Other PFT changes, such as N uptake, modified the plants' ability to compete with bacterial groups involved in N cycling.

Introduction

Terrestrial ecosystems can be divided into aboveground and belowground subsystems in which numerous interactions and feedbacks play crucial roles in ecosystem functioning (Grigulis et al. 2013; Manning et al. 2015). Plant-microbial relationships are at the heart of the interactions between these two subsystems (van der Heijden et al. 2008; van der Putten et al. 2016). About 80% of terrestrial plant species are colonized by AMF, mainly from the phylum Glomeromycota (Smith and Read 2010). The rate and extent of AM colonization differ with taxonomic group at Family level (Hart and Reader 2002). Root AMF colonization can be directly affected by the host plant regulating its carbon (C) allocation to roots, and can be indirectly mediated by changing soil properties (Urcelay et al. 2009). This symbiosis is characterized by an exchange of nutrients such as: phosphorus (P) and nitrogen (N) from the fungus, and C from the host, and plays a key role in ecosystem processes and properties by promoting plant biodiversity and plant productivity (van der Heijden et al. 1998; Smith and Read 2010). AMF can transfer N to their host (Buecking and Kafle 2015), even though the contribution of AMF to plant N uptake is usually smaller than its contribution to plant P uptake (Smith et al. 2011). The relative contribution of AMF to the total N nutrition of the host plant can vary considerably and is context dependent (Smith and Smith 2011; Mensah et al. 2015); notably influenced by soil N availability (Azcon et al. 2008; Bonneau et al. 2013). Conversely, AM fungi can modify how plants affect the rhizosphere by changing the quality and quantity of the rhizodeposition (Jones et al. 2004). Likewise, interactions between plants and free-living soil microbes including competition for N acquisition are thought to be critical controls of N dynamics in ecosystems (Harrison et al. 2008; Legay et al. 2013; Bell et al. 2015). Plants primarily take-up inorganic-N forms such as nitrate (NO_3^-) and ammonium (NH_4^+) (Miller and Bowman 2002; Harrison et al. 2008), which consequently deprives some microbes of their substrates: NH_4^+ for nitrifying organisms and NO_3^- for denitrifying microbes (Hart et al. 1994), directly influencing N-cycling in the plant-soil system.

Interactions between microbial assemblages, associated with plant root systems, and the rhizosphere, can be modulated by plant functional traits (Nuccio et al. 2013). The relevance of plant functional traits for plant-microbial relationships has been shown in studies across different ecosystems (Laughlin 2011; Legay et al. 2014a; Faucon et al. 2017) and at different scales, from the individual (Cantarel et al. 2015; Bell et al. 2015; Legay et al. 2016) to the plant community (Baxendale et al. 2014; Kastovska et al. 2015). Repeated measurements of the traits of individual plants over a period of time are relevant for describing species responses to environmental changes and their effects on ecosystem processes (Lavorel and Garnier 2002). These effects can be scaled up to the community level according to the relative abundance of each species (mass ratio

hypothesis) (Grime 2002; Garnier et al. 2004), or quantified by directly measuring functional traits at the community level (Gaucherand and Lavorel 2007; Prieto et al. 2015). Similar functional-trait-based approaches are being adopted for microbes (Krause et al. 2014) but, as individual traits cannot be measured, an alternative strategy is required to quantify emergent effects and responses at the microbial-community level. Potential nitrification and denitrification can be assessed through Potential Nitrification Activity (PNA), Potential Denitrification Activity (PDA) and Potential Nitrogen Mineralization (PNM) (Attard et al. 2011; Moreau et al. 2015). These enzymatic activities reflect soil microbes' ability to mineralize soil organic matter (Legay et al. 2016, Masunga et al. 2016) and root arbuscular mycorrhizal colonization intensity and frequency (Mouhamadou et al. 2011; Binet et al. 2013; Luo et al. 2014), so are commonly used as proxies for community-level microbial functional properties. In the field, these community-level microbial functional properties have been found to correlate with community-level plant traits (Laughlin 2011; de Vries et al. 2012; Legay et al. 2014a), and recent studies under controlled conditions have started to uncover the mechanisms underlying these relationships, including competition for inorganic N (Cantarel et al. 2015; Moreau et al. 2015; Legay et al. 2016), the AMF sensitivity of the host plant (Legay et al. 2016) and rhizodeposition patterns (Baptist et al. 2015; Mellado-Vazquez et al. 2016). These studies suggest that plant traits are highly relevant for analyzing the role of plant-microbial relationships in ecosystem functioning (Legay et al. 2014a).

Plant traits differ between individuals within a species, according to their stages of growth and development (Parrish and Bazzaz 1985), and due to local adaptation and phenotypic plasticity in response to environmental conditions (Nicotra et al. 2010). In addition, past research has highlighted the likely effects of soil microbial communities and the soil processes they mediate on plant trait plasticity (Goh et al. 2013). Changes in plant performance during growth can involve a switch in plant functional traits (Niinemets 2005; Mason et al. 2013), from traits typifying an exploitative strategy (e.g. high Specific Leaf Area and Leaf N Content – Wright et al. 2004) to a more conservative strategy (the opposite traits). However, very little is known about the consequences of these switches for soil microbiota, ecosystem processes and functioning; although they can presumably be identified through changes in plant N-uptake capacities and in root-exudation patterns (Mougel et al. 2006; Philippot et al. 2013; Bell et al. 2015).

In our previous study, we investigated the effects of three subalpine grasses, *Dactylis glomerata* (L.), *Bromopsis erecta* (Huds.) Fourr. (formerly *Bromus erectus* (Huds.)) and *Patzkea paniculata* (L.) G.H.Loos (formerly *Festuca paniculata* (L.) Schinz & Thell.), on both soil bacterial activities and mycorrhizal status (Legay et al. 2016). We determined how plant-microorganism interactions were affected by plant identity

through their functional traits and how fertilization could affect these interactions after three months of growth in a pot experiment. In the present study, the aim was to investigate the dynamics of plant-microorganism interactions during the first 3-months growth of *D. glomerata*. For this, we investigate the effects on soil biotic interactions of functional-trait changes linked to the growth of *D. glomerata*. We focused on various microbial functional properties: AMF root colonization rate and three N-cycling microbial enzymatic activities, PNM, PNA and PDA. *D. glomerata* was grown in a greenhouse, under controlled conditions, in its native subalpine soil, and under either low or high N availability. Plant traits and microbial functional properties were measured after one, two and three months of plant growth. *D. glomerata* is considered to be an exploitative species, since it has high specific root length and high rates of soil NO_3^- and NH_4^+ uptake (Grassein et al. 2015); allowing it to compete with soil microbes for N, for example by out-competing nitrifiers for NH_4^+ (Cantarel et al. 2015) and nitrate reducers for NO_3^- (Moreau et al. 2015; Moreau et al. 2019). *D. glomerata* is able to develop a high root biomass which produces large quantities of rhizodeposited C (Baptist et al. 2015). This rhizodeposition of organic compounds can stimulate denitrification (Henry et al. 2008) or soil organic matter mineralization (Kuzyakov & Xu 2013). Therefore, changes in root traits during plant growth are expected to affect microbial groups and their activities. Thus, we hypothesized that (i) high root N uptake would have a negative effect on nitrifiers and denitrifiers by increasing competition for NH_4^+ and NO_3^- respectively, (ii) increased root biomass would have a positive impact on denitrification and mineralization through the input of root exudates-C usable by denitrifiers and microbes mineralizing organic matter (PNM) and (iii) roots would be increasingly colonized by AMF during growth to improve nutrient acquisition as plants continue to grow.

Methods

Species selection and soil sampling

Soil and *D. glomerata* tussocks were sampled in subalpine grasslands located at the Lautaret Pass in the upper Romanche Valley of the Central French Alps (45.041°N 6.341°E, 1650–2000 m a.s.l.). These grasslands have been managed under light-to-moderate grazing for the last sixty years (Quetier et al. 2007). Native soil was sampled to 20-cm depth, air-dried and sieved at 5.6 mm. Two weeks before planting, soils were rewetted and mixed with perlite to limit soil compaction (1/5 perlite/soil). Initial soil physical and chemical properties following rewetting but prior to planting were: clay 30%; silt 46%; sand 24%; total carbon content 44.4 $g.kg^{-1}$; total nitrogen content 4.14 $g.kg^{-1}$; total phosphorus content 1.79 $g.kg^{-1}$; pH (H_2O) 5.5-6. Soil ammonium (95.31 ±4.01 µg $N\text{-}NH_4^+.g^{-1}$ dry soil) and nitrate (34.49±0. µg $N\text{-}NO_3^-.g^{-1}$ dry soil) concentrations were higher than in

the field (Robson et al. 2010; Legay et al. 2013), reflecting the end of the microbial flush following soil rewetting. Within the landscape species pool, *D. glomerata* can be described as an exploitative species (characterized by high SLA, leaf and root N contents) and its occurrence ranges from common to dominant in subalpine grasslands (Gross et al. 2007a). We thus choose *D. glomerata* for this experiment because of its fast growth capacity, its sensitivity to mycorrhizal fungal colonization (Gross et al. 2010) as well as its strong capacity to take up all inorganic N forms (Grassein et al. 2015). Briefly, three tussocks were sampled from the same grasslands and their roots were washed and cleaned of soil particles. Each tussock was vegetatively propagated on floating perlite at the University of Caen in a greenhouse (16 h day 20°C/8 h night 16°C) with additional light provided by high pressure sodium lamps (400W Philips SON T-PIA Agro). These gave 450-µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) measured at the top of the plants (LI-190 quantum sensor, LI-COR Biosciences, Lincoln, NE, USA). Plants were supplied with a nutritive solution renewed every week (see Grassein et al. 2015 for nutrient solution composition). Tillers were separated from each individual every 2-3 weeks (3-4 tillers stage), repeating this process to increase tiller number for the experiment. We controlled that produced tillers in these conditions were free from AM fungi in their roots. To standardize plant size, each individual tiller (10-15-cm height) was clipped to 6-cm in length for the aboveground parts and 4-cm for the belowground parts.

Experimental growing conditions

Homogeneous tillers were grown in cylindrical PVC pots (7-cm diameter, 16-cm height; 617-cm^{-3}) for three months between the end of January and the end of April. In each pot, two individual tillers (randomly selected from the clones of the three mother tussocks) were grown in 500-g of soil/perlite mix and arranged in a temperature-controlled glasshouse at 20 / 16 ± 2 °C (day / night) air temperature, and supplemental lighting (see above for details) with a 16/8-h photoperiod. At the beginning of the experiment, two fertilization treatments were created: half of the pots were fertilized with 50 kg N ha^{-1} (14 µg N g^{-1} of dry soil) in the form of urea-based slow-release N fertilizer (Osmocote®) to simulate the organic fertilization applied to mountain grasslands, and the other half of the pots were unfertilized.

In total 18 pots were planted, these pots were randomly assigned to a fertilization treatment (either fertilized or unfertilized) and intended harvest date (after 1, 2, or 3 months), and randomly arranged under the lamp frames in the greenhouse. Hence, there were three replicates of each combination of fertilization treatments and harvest dates. A further 6 unplanted pots were included in the experiment; half of them fertilized and the other half

unfertilized. All of these unplanted pots were sampled at the end of the experiment (T3) to compare the plant *vs.* no plant effects on soil and microbial functional properties. All pots were weighed and watered three times per week to keep soil moisture at 20 g water 100 g^{-1} dry soil. To control for edge effects, temperature gradients under the lamps and any other positional effects, the position of all pots was changed twice a week.

Nitrogen labelling and plant harvest

To obtain a time-course of the plant and soil interactions, we sampled at 1, 2 and 3 months after planting, hereafter called T1, T2 and T3 respectively. At T1 and T2, the soil sticking to harvested roots was shaken free and collected, but at T3 root density was so high that all the soil in each pot was considered to be under the influence of the rhizosphere. One day before each harvest, N labelling was applied as a solution of $^{15}NH_4{}^{15}NO_3$ (3.2-mg N per pot) 100% dual-labeled with ^{15}N to determine *D. glomerata* N-uptake. At harvest, aboveground traits, namely total aboveground biomass (ABM), SLA, leaf N and C concentrations (LNC and LCC), and leaf dry matter content were measured following the standardized protocols of Pérez-Harguindeguy et al. (2013). Roots were carefully washed and separated by floatation in tepid water using sieve stacks with different mesh sizes (4, 2 and 0.5-mm), and total fresh root biomass was split vertically into three equivalent aliquots of equal mass. This division of samples was done to avoid analyzing C and N contents of those roots scanned in water which could induce leaks of soluble nutrients. Then one aliquot was dried at 60°C and the two others were kept in an alcohol solution (ethanol 10%, acetic acid 5%) for analyses of root traits and arbuscular mycorrhizal colonization. For root traits, root length was determined using a flatbed scanner (Epson Expression 10000XL – WINRHIZO software – Regent Instruments Inc., Canada), fresh mass and dry mass after 72h at 60°C were recorded to calculate root dry matter content (RDMC – calculated as root dry mass / root fresh mass), specific root length (SRL – calculated as length of root / dry mass of root) and root biomass. Aboveground and belowground biomass were used to calculate the proportion of total plant mass allocated to roots (i.e. root mass fraction (RMF); Poorter et al. 2012). The dried leaf and root samples were ground to a fine powder for total ^{14}N, ^{15}N and C analysis using an isotopic ratio mass spectrometer (IRMS, Isoprime, GV Instrument).

The N uptake was calculated as described in Grassein et al. (2015) using natural ^{15}N abundance of atmospheric N_2 (0.36636 + 0.004 %) as reference. ^{15}N enrichment reflecting N uptake was calculated using an isotope mass balance equation : $Q^{15}N = (Ntot \times Ei)/Es$; where $Q^{15}N$ is N derived from N uptake for each organ by individual, Ntot is the total N content of a given replicate i, Ei (%) is the atomic ^{15}N excess and Es is the nutrient solution atomic ^{15}N excess. ^{15}N uptake was calculated by summing the ^{15}N content of each organ, expressed per gram of

dry mass. The aboveground and belowground dry mass produced per unit of N captured was calculated as an estimate of leaf and root N use efficiency (Gross et al. 2007b).

Soil sampling and assays of microbial activity

Following harvest, the soil samples from each pot (50-g of soil sticking to roots for T1 and T2 and all soils for T3) were sieved through a 5.6-mm mesh and weighed. An aliquot was air-dried, another was oven-dried for 1 week at 70°C to determine soil water content. The third aliquot was stored at 4°C until soil and microbial functional properties were measured (within 48h). Soil nutrient contents (NO_3^-N and NH_4^+-N) were measured from K_2SO_4 (0.5 M) extracts of the fourth aliquot (Jones and Willett 2006) using a FS-IV colorimetric chain (OI-Analytical Corp., TX, USA). The potential N mineralization (PNM) rates were estimated using anaerobic incubations of the fifth aliquot (in darkness for 7 days at 40°C), during which time organic N was mineralized and accumulated as NH_4^+-N (Wienhold 2007). The difference between NH_4^+ content before (t1) and after the incubation (t2) gave PNM = $[(NH_4^+\text{-}N)_{t2} - (NH_4^+\text{-}N)_{t1}]$/soil dry mass/7 days. Total microbial biomass was measured from microbial biomass N, using the chloroform- fumigation extraction technique (Vance et al. 1987). After fumigation, extractable N was measured using 0.5 M K_2SO_4 extracts. Microbial biomass N was calculated from the microbial N flush (the difference in N between fumigated and unfumigated soil) using a correction factor of 0.54 (Brookes et al. 1985).

Potential rates of nitrification (hereafter PNA) were estimated following Dassonville et al. (2011). Briefly, 3 g fresh soil from each pot was incubated under aerobic conditions (180 rpm, 28°C, 10 h) in 30 mL of a $(NH_4)_2SO_4$ solution (2 mg N L^{-1}). Rates of NO_2^- and NO_3^- production were measured after 2, 4, 6, 8 and 10 h by ionic chromatography (DX120; Dionex, Salt Lake City, UT, USA). PNA was calculated from the slope of the linear regression curve of NO_3^- plus NO_2^- production versus time. Potential denitrification activity (hereafter PDA) was determined according to Attard et al. (2011). Briefly, c. 10 g dw soil was placed at 28°C under anaerobic conditions using HeC_2H_2 (90:10) mixture inhibiting N_2O-reductase activity. Each flask was supplemented with c. 3-mL KNO_3 (50-mg $N\text{-}NO_3^-$ .g^{-1} dw), glucose (0.5-mg C g^{-1} dw) and sodium glutamate (0.5-mg C g^{-1} dw), topped-up with distilled water to reach the water-holding capacity. N_2O was measured after 2, 3, 4, 5 and 6 h, using a gas chromatograph (microGC RS3000; SRA instruments, Marcy l'Etoile, France). PDA was calculated from the slope of the linear regression curve of N_2O production versus time.

Arbuscular mycorrhizal colonization in root tissues was determined using the method developed by Phillips and Hayman (1970). Briefly, the percentage of mycorrhizal root colonization was estimated by visual observation of

fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v). Parameters of mycorrhizal fungal colonization were determined according to Trouvelot et al. (1986) using the MYCOCALC program (http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html). The colonization frequency (F%) is the ratio between colonized root fragments and total number of root fragments (called root AMF colonization frequency hereafter). It gives an estimate of the root length colonized by the fungus. The colonization intensity (M%) is an estimate of the amount of root cortex which became mycorrhizal (called root AMF colonization intensity hereafter). These two parameters were measured on individual plants before starting the experiment and at each harvest time.

Data analysis

The changes in plant traits, microbial functional properties and soil abiotic properties over the duration of the experiment, and effects of the fertilization treatments, and their interaction, were tested using two-way ANOVAs followed by least-squared difference post-hoc tests (Table 1). Preliminary statistical analyses found that fertilization had non-significant effects on all plant traits, and we found only one interaction between time and fertilization (for LCC - Table 1). The pattern was similar for soil abiotic parameters, root AMF colonization and microbial parameters (Supplementary Table 1). We found two possible explanations for these preliminary results: first, the rewetting of soil could have induced a microbial flush, stimulating N mineralization and, second, although we waited two weeks before planting, N availability was fairly high and could have dampened the effect of N addition. This fairly high N availability decreased to normal values (i.e. close to field condition) during the first month of the experiment. Furthermore, because of this fairly high initial N availability, it is likely that our N addition was insufficient to produce any noticeable treatment effect (Bardgett et al. 1999; Treseder 2008). However, NH_4^+ strongly decreased and all the NO_3^- was consumed by the end of the experiment, confirming that under these conditions, individual plants and N-cycling-related microbes were starving and in competition for inorganic N (see also Legay et al. 2016). Consequently, we focused our analysis on the effects of growing time on plant traits, microbial and soil properties, which were tested using one-way ANOVAs followed by least-squared difference post-hoc tests for pooled data at each time of harvest, giving three replicates with fertilization and three replicates without fertilization (n=6). At the final harvest (T3), the effects of plant presence on PNM, PDA and PNA were tested using a one-way ANOVA followed by least-squared difference post-hoc tests.

Lastly, we assessed whether mycorrhizal fungal colonization could modify the contribution of plant traits and/or soil abiotic properties to microbial enzymatic activities. We first used linear models to test whether changes in microbial enzymatic activities (PNM, PNA & PDA) and mycorrhizal fungal colonization parameters during the three months of the experiment were explained by the effect of plant-trait changes and/or by soil abiotic properties. Prior to analysis, the number of explanatory variables was reduced for plant traits using a principal component analysis (PCA), which allowed us to (i) visualize changes in plant traits during plant growth and to (ii) only keep the 10 plant traits contributing most to variation along the two main PCA axes (e.g. Specific Leaf Area, Leaf Nitrogen Content, Leaf C:N ratio, Leaf Dry Matter Content, Root Nitrogen Content, Root C:N ratio, Root Dry Matter Content, Specific Root Length, Root Mass Fraction and N uptake). To account for uncertainty in the model selection process, we then used a model averaging approach (Burnham and Anderson 2003), which fitted all possible models nested within the full model and ranked them on the basis of AIC; we calculated the percentage of variance explained in the most parsimonious model. The second step was to include mycorrhizal fungal colonization parameters (root AMF colonization frequency and root AMF colonization intensity) as explanatory variables in the model, to assess their contribution to the change in microbial enzymatic activities (PNM, PNA & PDA). All analyses were performed in R version 3.4.4, using the lsmeans (Lenth 2016) for least-squared difference post-hoc tests, the "PCA" function of the package FactoMineR (Le et al. 2008) and the 'dredge' function of the package MuMIn. All data were conformed with the assumptions of normality and homoscedasticity.

Results

Variation in plant traits during *D. glomerata* growth

During *D. glomerata* growth, plant traits changed at each successive harvest date, with the different positions of root and leaf traits in multivariate trait space revealing a switch in nutrient use strategies (Fig 1A). The first axis of the PCA separated individuals with high root mass fraction (RMF) and high C to N ratio in leaves (LCN) and roots (RCN) from individuals with low RMF, but high specific leaf area (SLA) and specific root length (SRL), and high N content in leaves (LNC) and roots (RCN). The second axis of the PCA separated individuals with high leaf C content (LCC), high N uptake ability, high root dry matter content (RDMC) and low leaf dry matter content (LDMC) from individuals with the opposite traits. The first axis clearly separated individuals harvested at different times; individuals from T1 had low root mass fraction and high LNC, RNC, SLA and SRL, and individuals from T3 had the opposite traits (Figure 1B). The trait values for individuals from T2 were

intermediate between those of T1 and T3. The second axis separated individuals from T2, with high LCC and N uptake capacity, from individuals from T1 and T3. These changes in plant traits over time, together with the lack of effect of the fertilization treatment, were consistent with the results of the ANOVAs (Table 1). Thus, the following paragraph describes changes in plant traits over time irrespective of fertilization treatment (n=6).

Throughout the period of growth, aboveground and root biomass of *D. glomerata* increased (Table 1 and Figure 2A). Changes in plant leaf and root traits followed the expected patterns during the growth, with both SLA and LNC being negatively correlated with leaf nitrogen use efficiency (LNUE), root C:N ratio, RDMC and RMF and positively related to SRL (Supplementary Figure 1 and Table 1). Thirteen plant traits changed significantly as *D. glomerata* grew, whereas four were unchanged (relative growth rate, leaf dry matter content, root carbon concentration, root diameter). Leaf and root C:N ratio as well as leaf and root N use efficiency increased month by month, whereas SLA, LNC and RNC decreased (Table 1). The RMF did not vary between T1 and T2 but increased between T2 and T3 (Fig. 2B), RDMC increased between T1 and T2, and thereafter remained unchanged (Fig. 2C), while SRL decreased only between T1 and T2 (Fig. 2D). Leaf C content and root N uptake followed distinct patterns with higher values at T2 than at both other harvest times (Fig 2E, 2F).

Root AMF colonization and potential enzymatic activities

AMF were absent from the roots at T0 when individuals were planted in the pots, but present in the roots at T1 and thereafter maintained a similar average root AMF colonization frequency and intensity through T2 and T3 (F= 46.11, p<0.001 – Fig. 3A; F= 5.04, p=0.009; Fig 3B). The root AMF colonization intensity was 12.50 ± 8.04 % at T1 and did not subsequently vary at T2 or T3.

Potential nitrogen mineralization (PNM), nitrification activity (PNA) and denitrification activity (PDA) were measured in planted soils every month. PNA remained low and did not change significantly during plant growth (F= 3.73, p=0.055; Fig. 4A), PNM decreased month by month (F=587.98, p<0.001 – Fig. 4C) while PDA increased between T1 and T2, but remained unchanged from T2 to T3 (F= 34.50, p<0.001; Fig. 4E). After three months of plant growth, we compared microbial biomass and potential enzymatic activities in planted and unplanted soils. The microbial biomass (F= 19.06, p=0.001; Table 2), PNA (F= 24.90, p<0.001; Fig. 4B) and PNM (F= 11.26, p=0.007; Fig. 4D) were significantly lower in the presence of plants, whereas PDA was significantly higher in the presence of plants (F= 12.42, p=0.005; Fig. 4F).

Relationships between plant traits, microbial functional properties and soil nutrient concentrations

Soil abiotic properties varied with the time of harvest (Table 2). Although patterns were not entirely consistent across sampling times, soil organic matter and microbial biomass tended to decrease during the experiment. There were large changes in soil NH_4^+ and NO_3^- concentrations during the 3 months of plant growth (Table 2). From T0 to T2, soil NO_3^- decreased significantly by a factor of at least 10 then remained stable. Soil NH_4^+ decreased drastically from the start of the experiment T0 to T1, and further decreased slightly between T1 and T3, confirming that NH_4^+, as well as NO_3^-, was used by plants and/or microbes during the experiment.

The contribution of plant traits and soil properties to changes in microbial enzymatic activities (PNM, PNA & PDA) and mycorrhizal fungal colonization parameters was studied using a model averaging approach. PNM was mainly negatively related to root C:N ratio which contributed 84.5% out of the 95.4% variation explained by the best model (Table 3). No variables related to soil properties were retained in our model to explain the variation of PNM. The potential nitrification activity (PNA) was not tested since there was no effect of plant growth (time effect) on PNA activity. Finally, specific leaf area and soil nitrate together explained more than 80% of the variation in potential denitrification activity (PDA). Concerning AMF parameters, our models only explained a small percentage of the variation in root AMF colonization frequency (34.7%) and intensity (37.7% - Table 4). The variation in these mycorrhizal parameters was only explained by plant traits: root C:N ratio and RMF for root AMF colonization intensity, and root C:N ratio and leaf N content for root AMF colonization frequency.

We tested whether root AMF colonization and intensity changed the contribution of plant traits or soil properties to the variation in microbial enzymatic activities explained by our models. Including these two parameters did not improve the model for PNM, but did increase the percentage of variance explained by the PDA models. In fact, the best model including AMF explained 88.2% of PDA variation, of which 39.7% was explained by root AMF colonization frequency (Table 3). All alternative models are available in the Table S2. In order to check potential interactions between the different groups of soil microbes, we also integrated the other microbial activities as predictors in the different models. Only marginal interactions were detected and, in each case, models obtained were less parsimonious (Table S2).

Discussion

We set up an experiment in which *D. glomerata* tillers were grown in pots containing native subalpine soil to study the impact of changes in its functional traits during plant growth on both N-related bacterial activities and patterns of root AMF colonization. We found that the dominant functional traits of *D. glomerata*

shifted from an initial suite of resource-exploitative traits (high SRL and LNC, and low RDMC) towards somewhat more-resource-conservative traits after 3 months of growth. This change to more-resource-conservative traits has already been reported for *D. glomerata* individuals cultivated over long periods (Personeni and Loiseau 2005; Picon-Cochard et al. 2012), and is in line with shifting patterns in suites of plant traits found across a variety of species (Niinemets 2005; Mason et al. 2013). We observed a decrease in root and leaf N concentration which were paralleled by an increase in N use efficiency of leaves and roots. In addition, these temporal variations in trait values were consistent with the reduction of soil inorganic N availability found after three months of growth, and the concurrent increase in plant biomass allocation to roots (RMF – from T2 to T3) and N use efficiency (RNUE – from T1 to T3). Hence, the rate of N uptake decreased during plant growth, but at the plant scale this was compensated by increased root biomass. This probably allowed a similar total inorganic N uptake per plant (Legay et al. 2014b), but could have changed the influence of roots on N availability at the rhizosphere scale. Such an increase in root biomass can have a large effect on ecosystem functioning by increasing plant C inputs into the soil (Blagodatskaya et al. 2014; Baptist et al. 2015; Herz et al. 2018), including making more labile C available to the soil microbial community (see Kuzyakov and Xu 2013).

Differences in root functional traits affect plant-microbe relationships (Legay et al. 2014a; Bardgett et al. 2014; Cantarel et al. 2015; Moreau et al. 2015). This outcome can result from differences in plants' abilities to uptake soil nutrients and to compete with microbial communities such as nitrifiers for NH_4^+ (Kaye and Hart 1997; Cantarel et al. 2015) or nitrate reducers for NO_3^- (Moreau et al. 2015). Roots can also affect microbial communities through rhizodeposition. The quality and quantity of rhizodeposited C may modify microbial community composition (Van der Krift et al. 2001) and N cycling (Henry et al. 2008) indirectly through the addition of labile C which may stimulate microbial enzymatic activities (Jones et al. 2004). Alternatively, rhizodeposits can serve as chemical inhibitors of microbial enzymatic activities such as nitrification (Subbarao et al. 2009) or denitrification (Bardon et al. 2014). In our study, these two opposing mechanisms may participate, via their affect on N cycling, in the relationship between *D. glomerata* and N-cycle-related microbes: namely potential N mineralization (PNM), potential nitrification (PNA) and denitrification activity (PDA). In confuting our hypothesis, PNM, which produces ammonium through the degradation of soil organic N (Wienhold 2007), decreased throughout the experiment and was lower in planted than in unplanted soils, suggesting that it is strongly negatively affected by *D. glomerata*. Yet, plant species are well-known to stimulate PNM (Blagodatskaya and Kuzyakov 2008), and more broadly the mineralization of organic matter (Kuzyakov and Xu 2013), while microbes are more efficient than plants in the uptake of organic N (Fischer et al. 2010). In our

study, although biomass allocation to roots increased as *D. glomerata* grew, and was probably paralleled by increasing rhizodeposited C (Baptist et al. 2015), we found that PNM and microbial biomass decreased over time. The root C:N ratio was negatively related to PNM and was the variable which best explained the variation in PNM (84.5%). Root C:N may influence the C:N ratio of the labile soil fraction (Fornara et al. 2011) and affect the quality of organic matter and consequently its rate of mineralization (Personeni and Loiseau 2004; Kuzyakov and Xu 2013). Another explanation is that many of the microbes able to mineralize N are also denitrifiers (Redondo-Nieto et al. 2013), and microbial denitrifying activity, may also have been promoted by plant rhizodeposits of labile C that microbes preferentially consume over C compounds obtained from mineralization of soil organic matter, or because root growth may have affected other soil properties such as oxygen availability (see the review of Moreau et al. 2019). In other words, change in root traits and related rhizospheric properties could have promoted denitrifying activity rather than mineralization of soil organic matter in those microbial groups able to realize both enzymatic activities (Redondo-Nieto et al. 2013; Shi et al. 2019).

According to our hypothesis, we found a higher PNA rate in the unplanted soil than in the presence of *D. glomerata*, and this value did not vary during plant growth. However, as PNA remained similar throughout the growth period, our experiment did not allow us to determine which plant traits affected PNA even though the presence of plants did decrease PNA activity. Additional experiments including more plant species to create a gradient of plant traits would be needed to determine whether the negative effect of plants on PNA found in this experiment is due to competitive interaction between nitrifiers and *D. glomerata* for NH_4^+, as previously reported for subalpine (Legay et al. 2014a; Grassein et al. 2015) and other plant species (Kaye and Hart 1997; Bardgett et al. 1999), or due to an inhibitory effect from plant rhizodeposits (Subbarao et al. 2009). Conversely to PNM and PNA, but in accordance with our hypothesis, there was an increase in PDA over time throughout the experiment, and a higher PDA in the planted than in the unplanted soils. We also found that specific leaf area (SLA) was negatively related to PDA and explained the most of its variation, and that soil nitrate was negatively related with PDA and explained a little of its variation. In other words, at the beginning of the plant growth (T0 to T1), *D. glomerata* had a more-exploitative strategy with low biomass but high SLA, high ability to uptake soil inorganic N and low leaf (LNUE) and root N use efficiency (RNUE), while PDA was low. This suite of plant traits caused a large reduction in soil NO_3^- and NH_4^+ from T0 to T2. Between growing time (T1 to T3), *D. glomerata* developed high leaf and root biomass, and with the decrease of soil N resources individuals switched to a more-conservative strategy with low SLA, high LNUE and RNUE, while over the same time period PDA increased. These results support the idea that *D. glomerata* and denitrifiers were in competition for soil N

resources (e.g. NO_3^-) at the beginning of plant growth when plants develop an exploitative strategy (high root N uptake, high SRL), as reported in previous studies (Grassein et al. 2015, Moreau et al. 2015). When plants reached the end of the growing period, with high root biomass and a more-conservative strategy (lower SRL, lower N uptake), they no longer competed with denitrifiers for NO_3^-, leading to an increase of PDA (Abalos et al. 2018, Moreau et al. 2019). These results are supported by those from Abalos et al. (2018) in which N uptake was higher in exploitative species than in conservative species, leading to lower N_2O emission as the result of lower denitrification activities. Although our experiment did not focus on this mechanism, our results suggest that, as for PNM, the increase in root biomass during plant growth modified soil properties to the benefit of denitrifiers: either through an increase in rhizodeposited C (Blagodatskaya et al. 2014; Baptist et al. 2015; Herz et al. 2018), which may in turn have fueled heterotrophic denitrifiers (Henry et al. 2008); or through lower oxygen partial pressure in the rhizosphere *via* increased root respiration and increased microbial activity (Woldendorp 1962; Smith and Tiedje 1979). In field conditions, grasslands dominated by exploitative species and soil dominated by bacteria have higher microbial activities and lower N retention (high N leaching and denitrification), whereas grasslands dominated by conservative species and soil dominated by fungi, and bacteria with slower activities, are associated with greater soil N retention (De Vries & Bardgett, 2016; Grigulis et al., 2013). These results are somewhat inconsistent with our findings, since we report the highest PDA under *D. glomerata* individuals with a more-conservative strategy and not under *D. glomerata* individuals with a more-exploitative strategy. However in the field patterns were mainly recorded from permanent grasslands where all plant species, whatever their strategy of nutrient conservation, are already well developed with root systems prospecting large volumes of soil. Moreover, inputs of plant litter can affect microbial community activities differently depending on plant species composition (Laughlin 2011). To test whether the switch from exploitative to conservative plant traits that we found in this study can occur in nature, grasslands sown with a mixture of plant species containing a wide range of trait values should be followed throughout the plant development cycle (from seed to mature plants) during one growing season.

During its growth, *D. glomerata* recruited soil AMF and its root colonization intensity was maintained throughout the experiment. Even though there was no significant change in root AMF colonization intensity from T1 to T3, the increase in root biomass over the same period suggested that active mycorrhizal symbiosis was maintained during growth and continued root AMF colonization may be required for nutrient acquisition. Indeed, as *D. glomerata* individuals grow larger they need more nutrients, thus a stable root AMF colonization rate should lead to a stable nutrient supply for the plant from the fungus per unit of plant biomass. Additional

experiments are needed to demonstrate the nutritional role of AMF associated with *D. glomerata* because we found that root AMF colonization was not associated with *D. glomerata* N uptake or with soil inorganic N concentration. There was only an indirect link between leaf N content or root C:N ratio and both root AMF colonization frequency and intensity. A high leaf N content or low root C:N ratio (and high root AMF colonization frequency and intensity) could be related to high N uptake by plants (Craine et al. 2003), and this suggests that root AMF presence could stimulate plant N uptake. The model explaining the drivers of PNM was not improved by including mycorrhizal variables associated with root AMF colonization suggesting that AMF which can provide N originating from soil organic N to plants did not affect ammonifiers in our study. The model explaining the drivers of PDA was the only one which was improved by including mycorrhizal variables associated with AMF colonization. In this model, SLA was always the best explanatory variable (48%) but root AMF colonization frequency (40%) also explained PDA variation. As explained above, even though our study was not designed to test the role of root AMF colonization in the relationships between plants and N-related microbial activities, our results still shed some light on these relationships. In fact, root AMF colonization frequency was positively correlated with PDA, which suggests that stable root AMF colonization during plant growth might be beneficial to PDA. Two issues need to be further explored: either root AMF colonization allows plants to uptake NH_4^+ from the soil avoiding competition with plant-denitrifiers for soil NO_3^-; or, C allocated by plants to their AMF symbionts also benefits denitrifiers (Kastovska et al. 2015) which are able to recycle C previously incorporated in roots, or may able to feed on C from fungal and root exudates (Bahn et al. 2013). Although, we did not find a relationship between AMF and PNA, we had no conclusive evidence to explain why PNA stayed stable and very low throughout the experiment. However, either of the two previous mechanisms we describe would explain the low PNA in our experiment. In fact, PNA might have suffered from competition with AMF for NH_4^+, as found in a recent study where AMF did not directly affect PNA but did influence the composition of the nitrifier community as the result of competition for NH_4^+ (Veresoglou et al. 2019).

Conclusions

As leaf and root biomass of *D. glomerata* increased, its growth was associated with a shift from an initial suite of resource-exploitative traits towards somewhat more-resource-conservative traits. These changes were consistent with a decrease in soil inorganic N, and concurrent increase of biomass allocation to the roots and N use efficiency. Plants of *D. glomerata* recruited AMF early during their growth and root colonization intensity was

maintained throughout the culture experiment suggesting that active symbiosis may contribute to nutrient acquisition for plant growth.

The shift in plant functional traits resulted in a lower SLA and SRL, reducing the ability of plants to acquire soil N. This shift resulted also in a higher allocation to root biomass (high RMF) and a change in root quality (high root C:N ratio); trends which are known to reflect changes in plant exudation patterns and consequently the quality of soil organic matter and the labile soil fraction. This plant-trait shift was consistent with a decrease in potential N mineralization (PNM) and an increase in potential denitrification activity (PDA). These patterns support the hypothesis that plants drive soil-N dynamics by: (i) changing their ability to uptake plant N and reducing their ability to compete with soil microbes (e.g. denitrifiers), (ii) by modifying the quantity and the quality of labile C resources and influencing the activity of N-cycling-related heterotrophic microbes. The contribution of root AMF colonization frequency to explaining PDA variation also supports this hypothesis, since denitrifiers can directly and/or indirectly benefit from the C allocated by plants to the AMF symbiont. To more deeply elucidate the functional role of AMF in our study, ^{15}N could be added to a soil compartment enclosed by a 25- to 35-µm nylon mesh allowing in-growth of hyphae but not roots. Nevertheless, the patterns of plant-AMF symbiosis that we describe support the idea that root AMF colonization may allow plants to uptake NH_4^+ in the soil avoiding plant-denitrifier competition for soil NO_3^- but at the expense of greater competition with nitrifiers, thus modifying the role of the plant in driving soil N cycling.

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Figure legends:

Figure 1. Principal components analysis (PCA) of plant functional traits of *Dactylis glomerata* (DG) under fertilized ("F_" individual) and unfertilized ("NF_" individual) conditions over three months of growth. The PCA represents (A) the projection of 17 plant traits and (B) the projection of *Dactylis glomerata* individuals from 17 plant traits data. Abbreviations: leaf dry matter content (LDMC), leaf carbon concentration (LCC), leaf C:N ratio (LC:N), leaf nitrogen concentration (LNC), relative growth rate (RGR), root biomass (RM), root carbon concentration (RCC), root C:N ratio (RC:N), root diameter (Diam), root dry matter content (RDMC), root mass fraction (RMF), root nitrogen concentration (RNC), aboveground biomass (ABM), shoot:root ratio (SRR), specific leaf area (SLA), specific root length (SRL).

Figure 2. Temporal dynamics of aboveground biomass (A), root mass fraction – RMF (B), root dry matter content – RDMC (C), specific root length – SRL (D), leaf carbon content (E) and nitrogen uptake rate (F) over three months (T1, T2, T3) of *Dactylis glomerata* growth (n=6, mean ± SE). The results of least-square-difference post-hoc tests are represented by different letters when significant differences ($p < 0.05$) between means were obtained. The absence of error bars reflects the very similar values between the six replicates meaning that SEs were too small to be visible.

Figure 3. Temporal dynamics of root AMF colonization frequency (A) and root AMF colonization intensity (B) at the beginning of the experiment (T0) and over three months (T1, T2, T3) of *Dactylis glomerata* growth (n=6, mean ± SE). The results of least-square-difference post-hoc tests are represented by different letters when significant differences ($p < 0.05$) between means were obtained. The absence of error bars reflects the very similar values between the six replicates meaning that SEs were too small to be visible.

Figure 4. Temporal dynamics of (A) potential nitrification activity (PNA), (C) potential nitrogen mineralization (PNM) and (E) potential denitrification activity (PDA) over three months (T1, T2, T3) of *Dactylis glomerata* growth and the effect of the presence or absence of this plant species (planted *vs* unplanted soils) on PNA (B), PNM (D) and PDA (F) (n=6, mean ± SE). The results of least-square-difference post-hoc tests are represented by different letters when significant differences ($p < 0.05$; ns: not significant) between means were obtained.

Supplementary Figure 1. Relationships between leaf functional traits (specific leaf area (SLA) and leaf nitrogen content (LNC)) and root functional traits (root C:N ratio, specific root length (SRL), root dry matter content (RDMC) and root mass fraction (RMF). Mean values (n=6) of *Dactylis glomerata* after one month (light gray), two month (dark grey) or three month (black symbols) of growth.

Table 1. Plant biomass and plant functional traits for each fertilization treatment and time of harvest. Values correspond to the mean of the three pots per treatments and harvest (n=3, mean ± SE, $p<0.05$). Values with different uppercase letters indicate significant differences between time of harvest (Time effect) and values with different lowercase letters indicate differences between treatments (Time x Fertilization effect) (Tukey post-hoc test).

	1 month (T1)		2 months (T2)		3 months (T3)		Time		Fertilization		Time x Ferti.	
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	*F*	*p*	*F*	*p*	*F*	*p*
Above-ground biomass (g)	0.53±0.08^{A}	0.76±0.03^{A}	1.61±0.19^{B}	1.85±0.11^{B}	2.41±0.14^{C}	2.58±0.26^{C}	**70.99**	**<0.001**	2.75	0.123	0.04	0.963
Root biomass (g)	0.28±0.06^{A}	0.41±0.04^{A}	0.89±0.14^{B}	1.10±0.14^{B}	1.78±0.05^{C}	1.93±0.12^{C}	**114.30**	**<0.001**	3.93	0.071	0.09	0.913
Relative Growth Rate (mg .d^{-1})	0.036±0.004	0.041±0.005	0.039±0.002	0.041±0.004	0.032±0.002	0.030±0.003	3.34	0.070	0.28	0.605	0.49	0.622
Shoot :Root ratio	1.95±0.13^{B}	1.87±0.09^{B}	1.82±0.08^{B}	1.71±0.13^{B}	1.35±0.05^{A}	1.33±0.08^{A}	**18.24**	**<0.001**	0.76	0400	0.09	0.911
Root Mass Fraction (g dry root .g^{-1} dry plant)	0.34±0.015^{A}	0.35±0.012^{A}	0.36±0.009^{A}	0.37±0.017^{A}	0.43±0.09^{B}	0.43±0.015^{B}	**21.80**	**0.001**	0.67	0.428	0.07	0.929
Leaf N Concentration (%)	4.54±0.03^{C}	4.40±0.08^{C}	2.63±0.35^{B}	2.75±0.23^{B}	1.66±0.05^{A}	1.85±0.34^{A}	**77.32**	**<0.001**	0.09	0.774	0.29	0.755
Leaf C Concentration (%)	44.85±0.21^{a}	45.82±0.45^{ab}	46.59±0.10^{b}	46.47±0.10^{b}	45.37±0.12^{a}	44.80±0.18^{a}	**23.14**	**<0.001**	0.28	0.608	**6.10**	**0.015**
Leaf C:N ratio	9.88±0.07^{A}	10.42±0.27^{A}	18.33±2.39 ^{B}	17.17±1.56^{B}	27.30±5.65^{C}	26.29±0.80^{C}	**20.46**	**<0.001**	0.065	0.080	0.07	0.937
Leaf dry matter content (mg.g^{-1})	299.9±14.4	290.1±6.9	298.4±11.9	288.5±7.9	293.8±3.0	318.8±5.0	1.19	0.337	0.05	0.821	2.44	0.129
Specific Leaf Area (m^{2}.kg^{-1})	25.64±0.40^{C}	27.24±0.25^{C}	19.03±1.13^{B}	20.40±0.47^{B}	17.89±0.78^{A}	16.37±0.24^{A}	**115.74**	**<0.001**	0.88	0.366	3.80	0.053
Root N concentration (%)	1.36±0.04^{C}	1.40±0.02^{C}	1.06±0.06^{B}	1.09±0.06^{B}	0.84±0.05^{A}	0.89±0.05^{A}	**56.79**	**<0.001**	1.07	0.322	0.05	0.949
Root C concentration (%)	44.49±0.36	43.09±1.36	43.41±0.80	42.80±0.70	42.94±0.28	43.83±1.17	0.31	0.738	0.28	0.608	0.88	0.438
Root C:N ratio	32.85±0.90 ^{A}	30.71±1.38^{A}	41.12±2.88^{B}	39.55±1.63^{B}	51.70±2.95^{C}	49.67±3.18^{C}	**33.09**	**<0.001**	1.01	0.330	0.01	0.99
Root dry matter content (mg.g^{-1})	138.9±7.6^{A}	137.3±1.5^{A}	168.6±1.9^{B}	160.2±1.4^{B}	155.2±4.1^{B}	153.9±7.3^{B}	**15.12**	**<0.001**	0.94	0.352	0.34	0.718
Specific Root Length (m.g^{-1})	109.67±10.8^{B}	105.54±1.36^{B}	74.03±8.92^{A}	68.15±5.84^{A}	67.25±2.89^{A}	72.14±2.95^{A}	**22.30**	**<0.001**	0.11	0.751	0.40	0.677
Root Diameter (mm)	0.53±0.02	0.53±0.00	0.53±0.03	0.54±0.03	0.56±0.01	0.54±0.01	0.71	0.514	0.01	0.946	0.53	0.599
Leaf N use efficiency (g dry mass .g-1 N)	22.03±0.13^{A}	22.73±0.39^{A}	39.32±5.06^{B}	36.94±3.28^{B}	60.17±1.73^{C}	58.78±12.88^{C}	**20.24**	**<0.001**	0.05	0.834	0.04	0.965
Root N use efficiency (g dry mass .g-1 N)	73.84±2.16^{A}	71.21±0.93^{A}	94.64±5.53^{B}	92.59±5.38^{B}	120.42±7.10^{C}	113.29±6.02^{C}	**38.90**	**<0.001**	0.92	0.356	0.15	0.860
N uptake rate (mmol N .g^{-1} root .h^{-1})	1.82±0.27^{AB}	2.22±0.16^{AB}	2.61±0.30^{B}	2.47±0.28^{B}	1.62±0.06^{A}	1.27±0.13^{A}	**12.64**	**0.001**	0.03	0.855	1.56	0.250

Table 2. Soil abiotic properties and microbial biomass at each harvest time, and with or without plants at the final harvest (T3). Values correspond to the mean of the six pots (mean of the fertilized and unfertilized treatments) for each plant treatment (with or without plant) and each time of harvest (n=6, mean ± SE, $p<0.05$). Values with different uppercase letters indicate significant differences between time of harvest (least-square-difference post-hoc test) and * indicate significant difference between pots with and without plants.

	T0	T1	T2	T3/Planted	Time effect		T3/Unplanted	Plant effect	
	0 months	1 month	2 months	3 months	*F*	*p*	3 months	*F*	*p*
Soil abiotic properties									
Soil Organic Matter content (%)	8.61 ± 0.11^{C}	7.53 ± 0.08^{A}	8.1 ± 0.11^{B}	7.61 ± 0.09^{A}	36.46	<0.001	7.61±0.07	0.002	0.967
Ammonium concentration (µg $N\text{-}NH_4^+ .g^{-1}$ dry soil)	95.31 ± 1.46^{C}	35.17 ± 3.42^{B}	26.71 ± 2.48^{AB}	25.68 ± 1.27^{A}	205.79	<0.001	58.85±3.04*	101.59	<0.001
Nitrate concentration (µg $N\text{-}NO_3^- .g^{-1}$ dry soil)	34.49 ± 0.35^{C}	17.06 ± 1.99^{B}	2.99 ± 1.26^{A}	1.91 ± 1.67^{A}	109.66	<0.001	83.34±11.99*	45.23	<0.001
Microbial biomass (µg biomass $.g^{-1}$ dry soil)	14.21 ± 0.14^{C}	6.16 ± 1.07^{AB}	7.63 ± 1.46^{B}	3.04 ± 0.96^{A}	21.15	<0.001	13.81±2.12*	19.06	0.001

Table 3. Influence of plant traits, soil and mycorrhizal properties on microbial enzymatic activities. Linear models followed by model averaging were computed for each microbial enzymatic activity. The estimated coefficients (Estimate) and standard errors (SE) are indicated for each variable.

	Plant traits and soil property models				Plant traits, soil and mycorrhizal property models			
Response variables	Retained variables	% explained	Estimate ± SE	*p*	Retained variables	% explained	Estimate ± SE	*p*
Potential N Mineralisation (PNM)	Root C:N ratio	84.50	-0.309 ± 0.10	0.010	Root C:N ratio	84.50	-0.309 ± 0.10	0.010
	Root Mass Fraction	6.98	-68.176 ± 14.79	<0.001	Root Mass Fraction	6.98	-68.176 ± 14.79	<0.001
	Specific Leaf Area	3.93	0.555 ± 0.020	0.015	Specific Leaf Area	3.93	0.555 ± 0.020	0.015
Potential Nitrification Activity (PNA)	Not tested				Not tested			
Potential Denitrification Activity (PDA)	Specific Leaf Area	75.61	-0.009 ± 0.00	0.007	Specific Leaf Area	48.53	-0.012 ± 0.00	<0.001
	Soil Nitrate	4.60	-0.003 ± 0.00	0.081	AMF frequency	39.73	0.002 ± 0.00	0.001

Table 4. Influence of plant traits and soil properties on AMF colonization parameters. Linear models followed by model averaging were computed for each fungal colonization parameter. The estimated coefficients (Estimate) and standard errors (SE) are indicated for each variable.

	Plant traits and soil property models			
Response variable	Retained variables	% explained	Estimate ± SE	*p*
Root AMF colonization Frequency	Root C:N ratio	16.98	-1.55 ± 0.79	0.069
	Leaf N Content	16.15	-14.98 ± 5.71	0.019
Root AMF colonization Intensity	Root Mass Fraction	35.31	165.66 ± 56.82	0.011
	Root C:N ratio	2.38	-0.72 ± 0.27	0.019

Supplementary Table 1. Soil abiotic parameters, root AMF colonization and microbial parameters measured at each harvest time for both fertilization treatments. Values correspond to the mean of the three pots per treatment at each harvest (n=3, mean ± SE, $p<0.05$). Values with different uppercase letters indicate significant differences between harvest times (Time effect) and values with different lowercase letters indicate differences between treatments (Time x Fertilization effect) (least square difference post hoc test).

	1 month (T1)		2 months (T2)		3 months (T3)		Time		Fertilization		Time x Ferti.	
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	*F*	*p*	*F*	*p*	*F*	*p*
Arbuscular Mycorrhizal Fungal parameters												
Root Mycorrhizal colonization Frequency (%)	62.8±3.6	60.0±11.5	63.3±8.8	89.6±0.4	73.4±12.3	73.2±2.2	1.98	0.180	1.42	0.256	2.03	0.175
Root Mycorrhizal colonization Intensity (%)	9.4±0.6	18.1±7.9	10.3±3.2	9.1±0.9	13.5±7.7	14.7±4.2	0.48	0.631	0.50	0.495	0.53	0.599
Microbial parameters												
Microbial biomass (µg biomass $.g^{-1}$ dry soil)	$6.58±2.31^{AB}$	$5.74±0.39^{AB}$	$5.46±2.40^{B}$	$9.8±0.35^{B}$	$4.59±1.46^{A}$	$1.49±0.22^{A}$	**3.91**	**0.049**	0.01	0.907	3.21	0.077
Potential N Mineralization (µg $N\text{-}NH_4^+ \cdot g^{-1} \cdot$dry soil $\cdot d^{-1}$)	$19.21±0.37^{C}$	$19.63±0.31^{C}$	$12.33±0.88^{B}$	$13.26±0.36^{B}$	$1.66±0.22^{A}$	$2.62±0.52^{A}$	**629.45**	**<0.001**	3.68	0.079	0.19	0.832
Denitrification enzyme activity (µg $N\text{-}N_2O$ $.g^{-1}$ dry soil $.h^{-1}$)	$0.30±0.02^{A}$	$0.30±0.01^{A}$	$0.41±0.02^{B}$	$0.43±0.01^{B}$	$0.40±0.02^{B}$	$0.45±0.02^{B}$	**42.15**	**<0.001**	3.72	0.078	1.30	0.308
Nitrification enzyme activity (µg $N\text{-}(NO_2^-+NO_3^-)$ .g-1 sol sec $.h^{-1}$)	0.117±0.023	0.093±0.034	0.056±0.012	0.037±0.012	0.093±0.028	0.123±0.033	3.73	0.055	0.05	0.834	0.69	0.519
Soil abiotic parameters												
Soil Organic Matter (%)	$7.54±0.16^{A}$	$7.52±0.08^{A}$	$8.09±0.15^{B}$	$8.12±0.11^{B}$	$7.56±0.19^{A}$	$7.66±0.19^{A}$	**10.57**	**0.002**	0.06	0.798	0.06	0.934
Ammonium concentration (µg $N\text{-}NH_4^+$ $.g^{-1}$ dry soil)	$39.56±5.14^{A}$	$30.78±3.54^{A}$	$27.36±4.53^{A}$	$26.07±3.16^{A}$	$25.48±1.19^{A}$	$25.89±2.56^{A}$	**4.19**	**0.042**	1.20	0.294	0.93	0.422
Nitrate concentration (µg $N\text{-}NO_3^-$ $.g^{-1}$ dry soil)	$19.9±2.66^{B}$	$14.22±2.19^{B}$	$2.40±1.33^{A}$	$3.57±2.42^{A}$	$0.08±0.05^{A}$	$3.74±3.24^{A}$	**28.60**	**<0.001**	0.02	0.880	2.34	0.138

Supplementary Table 2. Influence of plant traits, soil and microbial properties (AMF and microbial enzymatic activities) on microbial enzymatic activities. Alternative linear models (ΔAIC of 2) followed by model averaging were computed for each microbial enzymatic activity. The estimated coefficients (Estimate) and standard errors (SE) are indicated for each variable.

	Plant traits and soil property models				Plant traits, soil and mycorrhizal property models			
Response variables	Retained variables	% explained	Estimate ± SE	*p*	Retained variables	% explained	Estimate ± SE	*p*
Potential N Mineralisation (PNM)	No alternative model				Root C:N ratio	86.05	-0.330 ± 0.10	0.006
					Root Mass Fraction	3.55	-55.99 ± 16.30	0.004
					Specific Leaf Area	4.89	0.62 ± 0.02	0.007
					PNA	1.60	-14.53 ± 9.63	0.155
Potential Nitrification Activity (PNA)	Not tested				Not tested			
Potential Denitrification Activity (PDA)	Soil Organic Matter	4.68	0.045 ± 0.02	0.079	AMF frequency	39.73	0.001 ± 0.00	0.003
	Specific Leaf Area	75.61	-0.013 ± 0.00	<0.001	Specific Leaf Area	43.80	-0.012 ± 0.00	<0.001
					Soil Organic Matter	6.06	0.025 ± 0.02	0.201
	Soil Organic Matter	4.68	0.037 ± 0.02	0.133	AMF frequency	39.73	0.001 ± 0.00	0.003
	Specific Leaf Area	75.61	-0.009 ± 0.00	0.138	Specific Leaf Area	13.19	-0.009 ± 0.00	<0.001
	Soil Nitrate	2.96	-0.002 ± 0.00	0.006	Soil Nitrate	36.70	-0.001 ± 0.00	0.196
					AMF frequency	39.73	0.002 ± 0.00	<0.001
					Specific Leaf Area	45.63	-0.011 ± 0.00	<0.001
					PNA	4.79	-0.188 ± 0.11	0.123
Root AMF colonization Frequency	No alternative model							
Root AMF colonization Intensity	Root N Content	1.55	29.79 ± 10.95	0.016				
	Root Mass Fraction	37.41	182.94 ± 60.33	0.008				